

What is claimed is:

1. A peptide less than 40 amino acids in length having a cleavage site between a glutamic acid on the N-terminal side of the cleavage site and a non-polar or uncharged residue on the C-terminal side of the cleavage site and wherein the peptide is cleavable by an enzyme having the amino acid sequence of SEQ ID NO:8 and/or SEQ ID NO:9.
- 5 2. The peptide of claim 1 wherein the peptide comprises the amino acid sequence of SEQ ID NO:3 or SEQ ID NO:4.
- 10 3. The peptide of claim 1 wherein the peptide is of natural or synthetic origin.
4. The peptide of claim 1 wherein the peptide comprises a detectable label selected from the group consisting of  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^3\text{H}$ ,  $^{14}\text{C}$ ,  $^{35}\text{S}$ ,  $^{32}\text{P}$ ,  $^{33}\text{P}$ , a fluorescent dye, or a colorimetric indicator.
- 15 5. The peptide of claim 1 wherein the peptide comprises a fluorophore and a quencher or acceptor located at opposite ends of the cleavage site of the peptide.
- 20 6. The peptide of claim 4 wherein the peptide further comprises an affinity moiety located at opposite ends of the cleavage site of the peptide.
- 25 7. A method to identify a compound that inhibits Aggrecanase enzymatic activity comprising the steps of:

contacting a test compound, an Aggrecanase, and a peptide less than 40 amino acids in length wherein the peptide comprises a cleavage site between a glutamic acid on the N-terminal side of the cleavage site and a non-polar or uncharged amino acid residue on the C-terminal side of the cleavage site and wherein the peptide is cleavable by an enzyme having an amino acid sequence corresponding to SEQ ID NO:8 and/or SEQ ID NO:9; and

detecting cleavage of the peptide wherein inhibition of peptide cleavage in the presence of a test compound indicates compound inhibition of Aggrecanase enzymatic activity.

8. The method of claim 7 wherein the method is conducted in a single reaction vessel.

9. The method of claim 7 wherein the enzyme is selected from the group consisting of Aggrecanase-1 and -2.

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10. The method of claim 7 wherein the peptide is selected from the group consisting of SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6 and SEQ ID NO:7.

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11. The method of claim 7 wherein the peptide further comprises a detectable label selected from the group consisting of  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^3\text{H}$ ,  $^{14}\text{C}$ ,  $^{35}\text{S}$ ,  $^{32}\text{P}$ ,  $^{33}\text{P}$ , a fluorescent dye, or a colorimetric indicator.

12. The method of claim 11 wherein the peptide further comprises a fluorophore and a quencher or acceptor located at opposite ends of the cleavage site of the peptide.

15 13. The method of claim 7 wherein the contacting step further comprises a cell expressing the Aggrecanase.

14. A method to detect the ability of a compound to inhibit Aggrecanase-1 or -2 enzymatic activity comprising the steps of:  
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contacting a test compound, an Aggrecanase secreted by a cell, and a peptide having an amino acid sequence selected from the group consisting of SEQ.ID.NO.:3 or SEQ.ID.NO.:4;  
incubating the compound, enzyme, and peptide to permit enzymatic cleavage of the peptide; and  
measuring enzymatic cleavage of the peptide wherein the method is conducted in a single reaction vessel without further manipulation.

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15. The method of claim 14 wherein the peptide comprises a detectable label selected from the group consisting of  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^3\text{H}$ ,  $^{14}\text{C}$ ,  $^{35}\text{S}$ ,  $^{32}\text{P}$ ,  $^{33}\text{P}$ , a fluorescent dye, or a colorimetric indicator.

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16. The method of claim 14 wherein the peptide comprises a fluorophore and a quencher or acceptor located at opposite ends of the cleavage site of the peptide.

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17. A method to identify a compound capable of inhibiting Aggrecanase activity comprising the steps;

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providing a peptide comprising an affinity moiety, an amino acid sequence selected from a group consisting of SEQ.ID.NO.:3 or SEQ.ID.NO.:4 and a detectable label, said affinity moiety and label located on opposite sides of a cleavage site encoded by the amino acid sequence;

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contacting the peptide with an affinity capture coated solid phase support for sufficient time to bind a portion of the peptide;

washing the support to remove unbound peptide;

contacting a solution comprising a test compound and functional enzyme with the peptide bound solid phase support for sufficient time to allow enzymatic cleavage of the peptide, thereby releasing the peptide and detectable label into the solution; and

measuring changes in the quantity of the detectable label as a result of compound modulation of expected enzymatic function.

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18. The method of claim 17 wherein the enzyme is selected from the group consisting of Aggrecanase-1 and -2.

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19. The method of claim 17 wherein the peptide comprises a detectable label selected from the group consisting of  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^3\text{H}$ ,  $^{14}\text{C}$ ,  $^{35}\text{S}$ ,  $^{32}\text{P}$ ,  $^{33}\text{P}$ , a fluorescent dye, or a colorimetric indicator.

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